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Lack of Barrels in the Somatosensory Cortex of Monoamine Oxidase A–Deficient Mice: Role of a Serotonin Excess during the Critical Period

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Summary

In a transgenic mouse line (Tg8) deficient for the gene encoding monoamine oxidase A (MAOA), we show that the primary somatosensory cortex (S1) lacks the characteristic barrel-like clustering of layer IV neurons, whereas normal pattern formation exists in the thalamus and the trigeminal nuclei. No barrel-like patterns were visible with tenascin or serotonin immunostaining or with labeling of thalamocortical axons. An excess of brain serotonin during the critical period of barrel formation appears to have a causal role in these cortical abnormalities, since early administration of parachlorophenylalanine, an inhibitor of serotonin synthesis, in Tg8 pups restored the formation of barrels in S1, whereas inhibition of catecholamine synthesis did not. Transient inactivation of MAOA in normal newborns reproduced a barrelless phenotype in parts of S1.

Introduction

Sensory cortices contain systematic representations of peripheral receptors. To understand how these cortical maps are formed and refined during development, the primary somatosensory cortex (S1) of rodents is an extensively used model because discrete cytoarchitectonic units in layer IV, the barrels, are isomorphically related to peripheral receptors, and their assembly delineates the entire body representation (Woolsey and Van der Loos, 1970; Welker, 1976; Wallace, 1987). The emergence of the primary sensory map results from an interplay between intrinsic and extrinsic factors (reviewed by O'Leary et al., 1994). Layer IV neurons in S1 have been shown to express intrinsic markers independently of the incoming afferents (Cohen-Tannoudji et al., 1994), whereas the formation of barrels depends on signals arising from the peripheral receptors via central relays in the trigeminal and thalamic nuclei. During development, topological organization follows a peripheral to central sequence along the somatosensory pathway (Belford and Killackey, 1979; Agmon et al., 1993, 1995). Lesions or genetic modifications of the peripheral receptors (Belford and Killackey, 1980; Jeanmonod et al., 1981; Welker and Van der Loos, 1986) or interruptions of the trigeminal sensory pathway (Jensen and Killackey, 1987) modify the pattern of cortical barrels.

Besides the specific thalamic afferents, cortical afferents from the brainstem or the basal forebrain are thought to have a modulatory role in the maturation of cortical maps (Kasamatsu et al., 1979; Bear and Singer, 1986; Gu and Singer, 1995). The monoaminergic afferents, serotonin (5-HT), noradrenaline (NA), and dopamine, all reach the cortex during embryonic development (Verney et al., 1982; Wallace and Lauder, 1983), before the cytogenesis of the cortex is achieved. In rodents, there is a transient postnatal hyperinnervation of the primary sensory cortices with NA-containing fibers (Lidov et al., 1978) and 5-HT-containing fibers (Fujimiya et al., 1986; D'Amato et al., 1987). The 5-HT afferents form periphery-related patterns, delineating barrels in S1 very early in development (Rhoades et al., 1990), coinciding with the clustered ingrowth of thalamocortical fibers (Blue et al., 1991), which transiently contain serotonergic receptors of the 5-HT_{1B} subtype (Bennett-Clarke et al., 1993). This sustained the idea that 5-HT and NA afferents, arising from the raphe nuclei and the locus coeruleus, respectively (Ungerstedt, 1971; Lindvall and Björklund, 1978), could have a supportive role in the formation of the thalamocortical projections or act upon the cytoarchitectonic maturation of the cortical neurons into barrels. In previous investigations, monoaminergic fibers were lesioned perinatally, using neurotoxins selective for the different monoamines (Loeb et al., 1987; Blue et al., 1991; Bennett-Clarke et al., 1994; Osterheld-Haas et al., 1994). However, since none of these lesions altered the normal pattern of barrels in the somatosensory cortex, it was concluded that neither NA nor 5-HT is essential to pattern formation in S1, although they could have trophic effects and be involved in modulating the plasticity of the cortical maps.

The effect of increased amounts of 5-HT on barrel field formation has not yet been considered. We examined this situation in the transgenic mouse line Tg8, in which the gene encoding monoamine oxidase A (MAOA) was serendipitously disrupted by the integration of an interferon-β transgene (Cases et al., 1995). MAOA and MAOB are two related enzymes that catalyze the oxidative deamination of monoamines, differing in their preferred substrate specificity (reviewed by Weyler et al., 1990). In the brain of Tg8 pups, there is a 700%–900% increase in the amount of 5-HT with a decrease of the 5-HT metabolite 5-hydroxyindoleacetic acid, during the first postnatal week, whereas NA is increased by 35%–70% (Cases et al., 1995). Later during development, there is a normalization of the 5-HT and 5-hydroxyindoleacetic acid amounts in Tg8 mice, owing in part to a compensation of the MAOA deficiency by MAOB. In the first description of this Tg8 mouse line, we reported the existence of permanent cytoarchitectonic alterations in the somatosensory cortex (Cases et al., 1995).

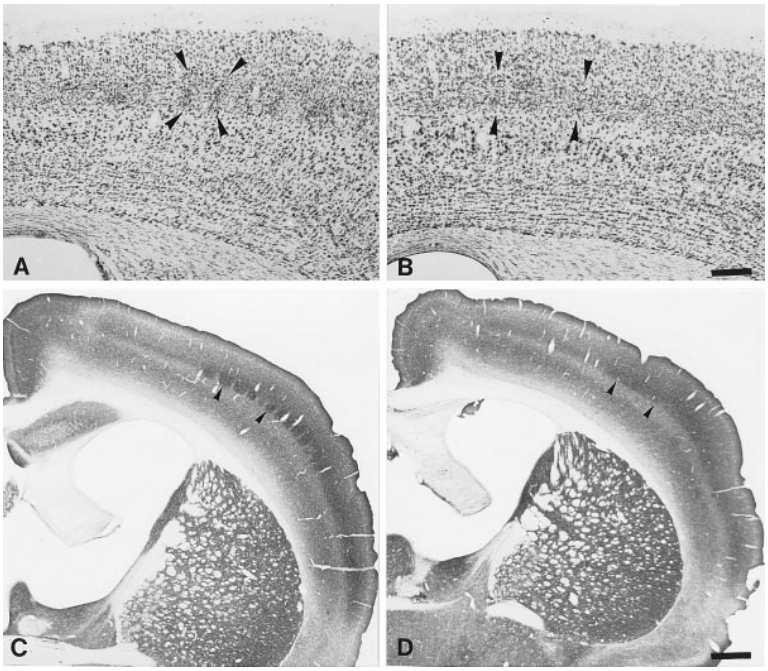

Figure 1. Lack of Barrels in the Primary Somatosensory Cortex of Adult Tg8 Mice

(A and B) Nissl staining; (C and D) cytochrome oxidase (CO) activity. The normal barrels in C3H mice are visible as clustered granular cells in layer IV, surrounding a cell-sparse hollow (arrowheads delimit one barrel in [A]) or as blobs of increased CO activity (arrowheads point to individual barrels in [C]). In Tg8 mice, granular neurons (B) and an increase in CO activity (D) form a continuous band in layer IV (delimited by arrowheads). Bars, 200 μm (A and B), 416 μm (C and D).

In the present report, we describe these cytoarchitectonic abnormalities by using different markers of the cortical cells and by labeling the serotonergic and thalamic cortical afferents. We demonstrate that similar permanent alterations can be caused by transient inhibition of MAOA activity during the first week of life in normal mice. Finally, we provide evidence that excessive brain 5-HT amounts are responsible for these alterations because in Tg8 mice inhibition of 5-HT synthesis but not of catecholamines restores the normal development of the barrel field.

Results

Abnormal Organization of the Cortical Barrel Field in Tg8 Mice: Neuronal and Glial Cortical Markers

In S1, the structural differentiation of layer IV neurons into barrels is visible with Nissl stain as cylindrically shaped aggregates. Barrels consist of a dense ring of granular neurons surrounding a paler, hollow area of lesser cell density and are separated by septae. Their assembly forms the barrel field (Woolsey and Van der Loos, 1970) (Figure 1A). In Tg8 mice, this cytoarchitectonic organization was not observed. Instead, the granular cells of layer IV formed a continuous band with homogeneous density (Figure 1B). As measured in 3-month-old mice at the level of the posteromedial barrel subfield (PMBSF), the thickness of layer IV was less in Tg8 mice (80 ± 5 μm, mean ± SEM from 2 cases) than in normal (C3H) mice (110 ± 10 μm). In other granular cortices (visual and retrosplenial), the thickness of layer IV was unaltered. The total cortical thickness was similar in Tg8 (840 ± 30 μm) and C3H mice (880 ± 25 μm).

Cytochrome oxidase (CO) histochemistry allows the visualization of neurons with heightened metabolic activity in the center of the cortical barrels (Figure 1C). At this level, CO is localized mainly in dendrites of cortical neurons, but also in axon terminals (Wong-Riley and Welt, 1980). In Tg8 mice, the intensity of CO activity was like that of C3H mice, but it did not form the characteristic barrels (Figure 1D). S1 was reconstructed in flattened preparations of the cortex that were tangentially sectioned and stained for CO. As previously described by Wallace (1987), the principal domains of S1, corresponding to the mystacial vibrissae (PMBSF), the anterior snout, the lower lip, the forepaw, and the hindpaw, could be identified in C3H mice (Figure 2g). Each element of this representation contains barrels or thin stripes (in the lower lip representation) that stain densely for CO. In Tg8 mice, the somatosensory map was profoundly modified: the mystacial vibrissae and the anterior snout representations were fused, with separations maintained only between the anterior snout, the lower lip, the forepaw (Figure 2h), the trunk, and the hindpaw (data not shown). These separations individualize the main domains of S1: those corresponding to the trigeminal nerve roots (infraorbital and supraorbital) and to the lemniscal afferents (limbs and trunk). Occasionally, barrel rows or a few of the larger caudal barrels were present in the PMBSF of Tg8 mice (visible on the 5-HT-stained preparation in Figure 4b). However, the septae had a blurred appearance in comparison with the sharp delimitation in the PMBSF of controls. Alterations were similar in postnatal day 8 (P8) pups and adult mice. Homozygous Tg8 females and hemizygous Tg8 males, born from homozygous or heterozygous mothers, displayed similar alterations, whereas heterozygous females displayed a normal-appearing barrel field (the *MAOA* gene is present on the X chromosome only).

The total area occupied by S1, as identified by the central area with heightened CO activity, was increased by 20% in Tg8 mice. This was determined from tangential preparations of P8 pups using camera lucida reconstructions of serial sections. The external contour of S1

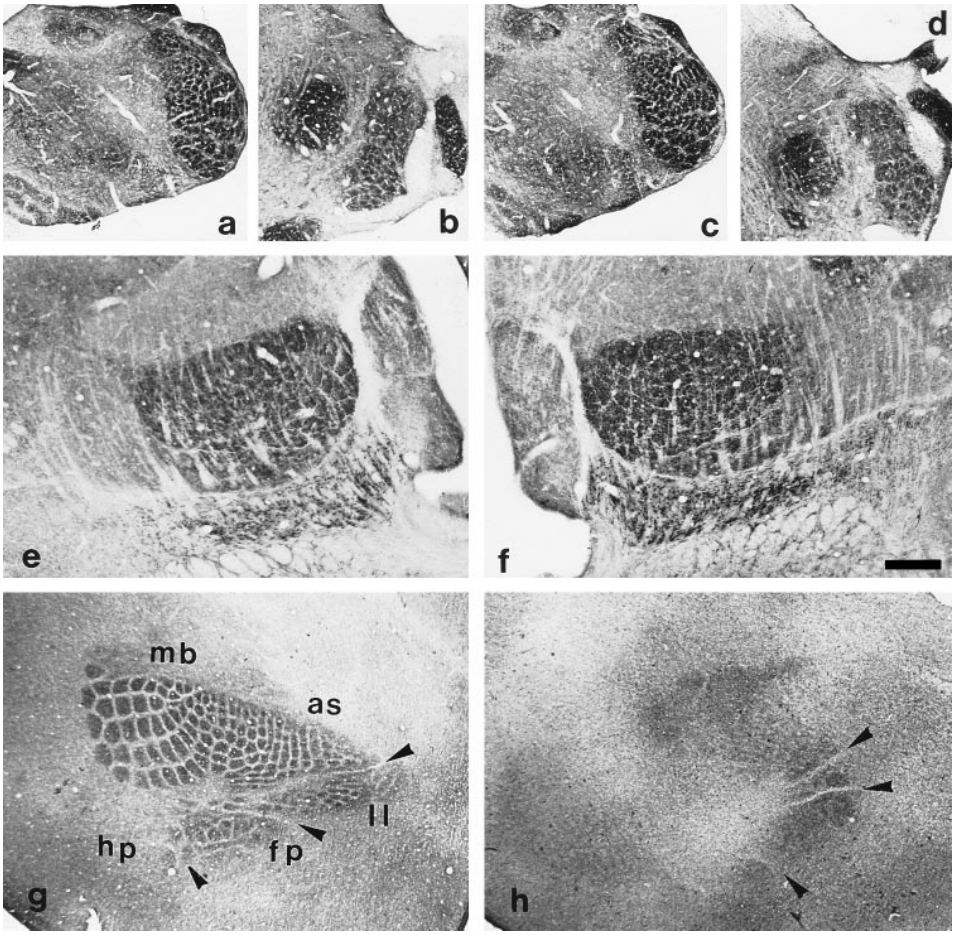

Figure 2. Altered Patterning in the Cortical Somatosensory Map Contrasts with the Normal Patterning in Lower Stations of the Somatosensory Pathway

Different relays of the somatosensory pathway as shown with CO activity in P7 C3H (a, b, e, and g) and Tg8 (c, d, f, and h) mice. Barrelettes have comparable staining and distribution patterns in C3H and Tg8 mice in the nucleus principalis of the trigeminalis (a and c) and in the nucleus oralis of the trigeminal complex (b and d); barreloids are normally stained and distributed in the ventrobasal thalamic nucleus (e and f). Flattened preparations of the tangentially cut cerebral cortex (g and h) show the different regions of the somatosensory representation: the large mystacial vibrissae (mb), the anterior snout (as), the lower lip (ll), the forepaw (fp), and the hindpaw (hp); these regions are separated by large septae (arrowheads) in both C3H and Tg8 mice. These main divisions of S1 are subdivided into a number of barrels in C3H (g) but not in Tg8 (h) mice; rostral is to the right and ventral is up. Bar, 370 μm (a–d), 260 μm (e and f), 650 μm (g and h).

was delineated and the enclosed area was measured (4.33 ± 0.16 mm^2 in C3H mice, $n = 4$; 5.28 ± 0.46 mm^2 in Tg8 mice, $n = 6$; $p = .1$ in the two-sample t test). No other obvious differences of brain or body growth were detected; body and brain weights were comparable in C3H and Tg8 pups, as was the total cortical area measured from the flattened hemispheres (59.7 ± 2.4 mm^2 in C3H mice, $n = 4$; 55.7 ± 6.7 mm^2 in Tg8 mice, $n = 6$).

A number of extracellular matrix molecules, such as the glycoprotein tenascin, are localized on the transient glial and glycoconjugate boundaries that surround the developing barrels in S1 (Crossin et al., 1989; Steindler et al., 1990), and it has been suggested that these proteins may be involved in the formation or stabilization of these cytoarchitectonic units, because of the temporal coincidence of their expression with the period of barrel formation. In P8 Tg8 pups, tenascin immunoreactivity was absent from layer IV, whereas it clearly delineated the barrel boundaries in C3H pups (Figures 3c and 3d). Tenascin immunoreactivity was normally distributed in other brain regions (Figures 3a and 3b).

Thus, markers of cortical cells, both neurons and glia, display an abnormal pattern of distribution in layer IV of S1.

Normal Patterning in Lower Stations of the Somatosensory Pathway

The patterned organization of the rodent somatosensory cortex has been shown to be tightly dependent on the organization of peripheral somatic receptors (reviewed in Van der Loos et al., 1991). Central relay stations in the brainstem trigeminal complex and the ventrobasal complex of the thalamus (VB) are necessary to convey this information from the periphery to the cortex. In each, patterned inputs and discrete cell clusters form topological replicas of the facial whiskers: the "barrelettes" in

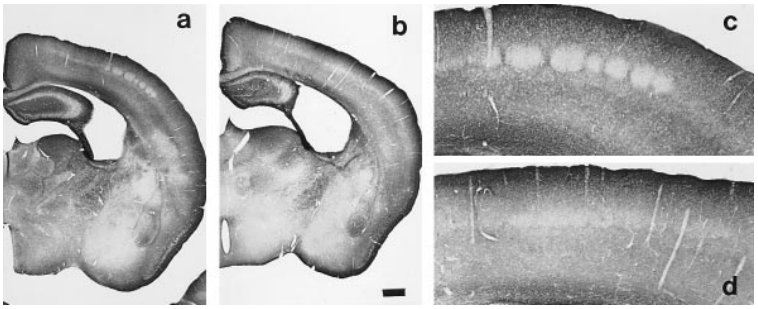

Figure 3. Lack of Tenascin-Labeled Glial Boundaries in Layer IV in Tg8 Pups

Tenascin immunostaining in C3H (a and c) and Tg8 (b and d) pups (P8) at low (a and b) and high (c and d) magnifications. Abnormal distribution of tenascin is visible in the somatosensory cortex of the Tg8 mouse surrounding a narrow, clear band in layer IV (d), instead of the normal staining that outlines the barrels (c). Tenascin immunoreactivity appears to be distributed normally in other brain regions. Bar, 0.4 mm (a and b), 125 μm (c and d).

the three subnuclei of the trigeminal complex (Ma, 1993) and the "barreloids" in the VB (Van der Loos, 1976).

In Tg8 mice, we observed CO-labeled patterns identical to those of C3H mice, in the VB (see Figures 2e and 2f) and in the different subnuclei of the brainstem trigeminal complex, as shown for the nucleus principalis (see Figures 2b and 2d) and interpolaris (see Figures 2a and 2c). Barreloid patterning in the VB was also visible at P8 with fluorescence (autofluorescence; Agmon et al., 1995) and was outlined with tenascin immunoreactivity. The vibrissae follicles in the mystacial pad had similar histological organization in C3H and Tg8 mice. Whisker length was not modified. The vibrissae-placing test in pups (Roubertoux et al., 1992) appeared to be normal in Tg8 mice.

The "barrelless" phenotype in Tg8 mice thus seems to be restricted to the cerebral cortex.

Permanent Alterations of the Thalamocortical Afferents

Since cortical afferents, particularly thalamocortical afferents, but possibly also serotonergic afferents, could have important roles in the lack of barrels, we looked for abnormalities in the distribution of these afferent axons.

Taking advantage of the fact that 5-HT_{1B} receptors are transiently expressed in the developing thalamocortical fibers of rats during the first postnatal week (Bennett-Clarke et al., 1993), we labeled these projections with the 5-HT_{1B} receptor ligand [^{125}I]cyanopindolol. In C3H mice, [^{125}I]cyanopindolol densely labeled S1, and the normal barrel field pattern could be seen in layer IV (Figures 4a and 4c); furthermore, dense binding was present over the ventral thalamus. In Tg8 mice, [^{125}I]cyanopindolol binding was reduced overall but was still visible in layer IV of S1, forming a continuous line instead of the normal disjunctive pattern (Figure 4b). In adults, comparable levels of [^{125}I]cyanopindolol binding were observed in Tg8 and C3H mice (Figures 4d and 4e). The distribution pattern of the binding sites was similar to that previously described in rats (Boschert et al., 1994), with no labeling in the thalamus or S1.

In adult mice, thalamocortical axons were labeled with dextran–biotin by iontophoretic injections of the tracer in the VB. The characteristic clustering of these thalamocortical fibers within the barrels was visible in C3H mice (Figure 5a), whereas in Tg8 mice the VB thalamic fibers arborized as a continuous band in layer IV (Figure 5b). This arborization had a preserved laminar distribution, remaining within the confines of the granular layer IV and with terminal arbors of a similar tangential extent in layer VI. Injections in two different rostrocaudal positions in the VB (see Experimental Procedures) resulted in a comparable topographical distribution of labeled fibers in Tg8 and C3H cortex (data not shown), suggesting that the general topography of the thalamocortical projection is not altered. In some cases, the injection site involved the posterior thalamic complex, resulting in a different type of cortical labeling; these nuclei send out axons over a wider tangential domain than the VB axons, with a different laminar location, in layer I and in the upper part of layer V, accumulating in the lower part of the septae of layer IV (Figure 5c) (Caviness and Frost, 1980; Chmielowska et al., 1989). In Tg8 mice, posterior thalamic fibers formed a continuous band just below

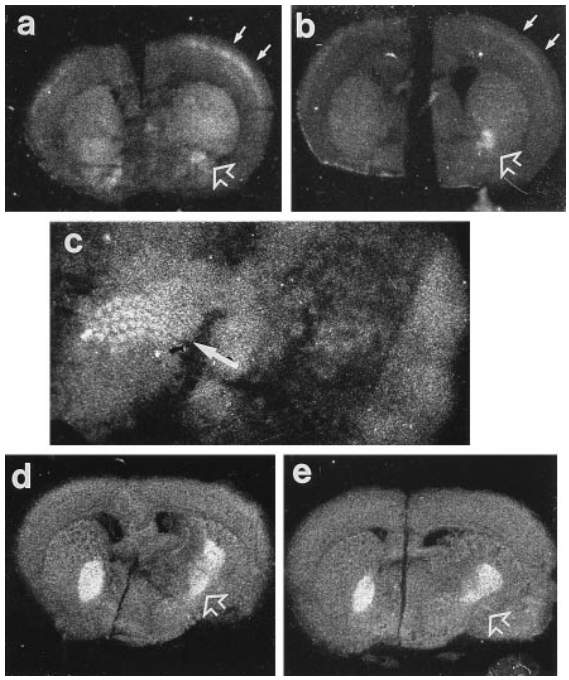

Figure 4. 5-HT_{1B} Binding Transiently Labels Thalamocortical Afferents in Mouse Pups, Showing Their Altered Distribution in the Tg8 Mice

Labeling of 5-HT_{1B} with [^{125}I]cyanopindolol at P8 (a–c) and in adults (d and e). A dense labeling of layer IV (arrows) with clustered distribution is visible in C3H mice at P8 on coronal (a) and tangential (c) sections; this binding is reduced in Tg8 mice and forms a uniform band (b). In adults, cyanopindolol binding is indistinguishable in C3H (d) and Tg8 (e) mice and has disappeared from the cerebral cortex.

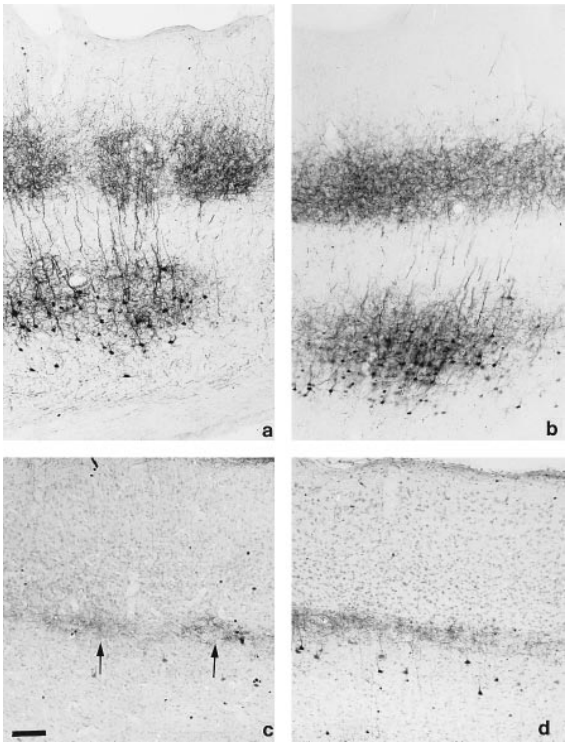

Figure 5. Altered Tangential Distribution of Thalamocortical Axon Terminals in Tg8 Mice

Dextran–biotin injections in the thalamus of adult C3H (a and c) and Tg8 (b and d) mice. Dextran–biotin was injected in the VB (a and b), labeling fibers that arborize in the barrel hollows in C3H mice (a) and within the barrelless layer IV in Tg8 mice (b). Injections involving the posterior thalamic complex label fibers just below layer IV (c and d), which accumulate at the level of barrel septae in C3H mice (c, arrows) but form a continuous line in Tg8 mice (d). Bar, 65 μm.

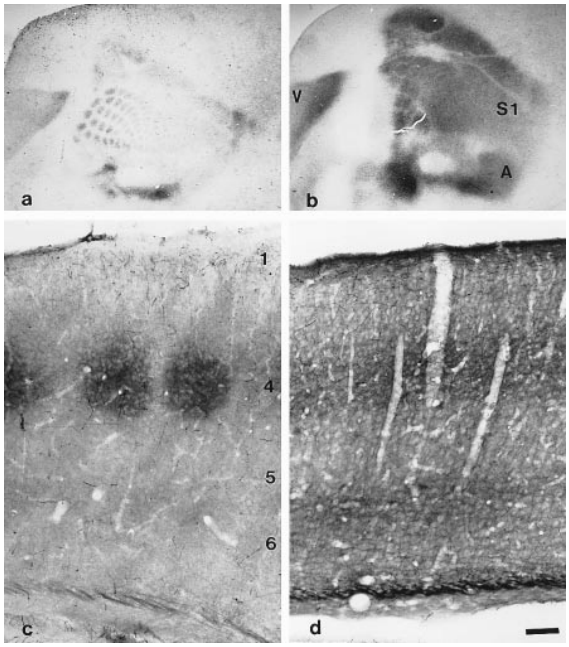

Figure 6. Altered Patterns of 5-HT-Labeled Fibers in the Cortex of Tg8 Pups

Increased 5-HT immunostaining is visible in the cerebral cortex of P8 Tg8 mouse pups (b and d) in comparison with C3H pups (a and c). On a tangential section through the flattened cortex of a C3H pup (a), the normal 5-HT innervation delineates the primary sensory cortices—visual (V), auditory (A), and somatosensory (S1)—with a barrel pattern in the latter. In Tg8 pups (b), 5-HT labeling is denser and more widespread, and lacks the normal patterning in S1. In coronal sections through S1 in C3H pups (c), dense accumulations of fine 5-HT fibers are visible in layer IV in the cortical barrels, with light labeling in layer VI. In matched sections from Tg8 pups (d), 5-HT immunoreactivity increases in all cortical layers, with enhanced background labeling. Bar, 0.8 mm (a and b), 65 μm (c and d).

layer IV that did not penetrate within layer IV (Figure 5d), but these fibers had a normal distribution in layer I.

These observations indicate that the normal distribution pattern of thalamic inputs to S1 is permanently modified, but this abnormality appears limited to its lack of normal tangential clustering and does not affect the general topography or laminar distribution of the terminal axons.

Transient Abnormalities of Serotonergic Afferents

In the cortex of Tg8 pups, 5-HT immunostaining was considerably increased, and labeled fibers were distributed abnormally (Figure 6). These features disappeared with age. In adult Tg8 and C3H mice, the cortical 5-HT innervation was identical (data not shown).

The qualitative abnormalities in the distribution of 5-HT fibers (observed even with lower dilutions of the 5-HT antiserum) can be understood better in the perspective of the normal developmental pattern of the cortical 5-HT innervation. The 5-HT fibers reach the cortex early in the embryonic period (Wallace and Lauder, 1983) but have a protracted postnatal development. During the first 2 postnatal weeks, there is a dense 5-HT innervation in S1 and auditory and visual cortices, which subsequently disappears. In S1, this transient innervation consists of accumulations of 5-HT-containing fibers within the barrel centers in layers IV and VI (see Fujimiya et al., 1986, for a description in mice; see D'Amato et al., 1987; Rhoades et al., 1990, for descriptions in rats). In Tg8 mice at P8, the distribution of 5-HT fibers within S1 differed strikingly from that of controls. In tangential sections, 5-HT immunoreactivity was more intense and covered a wider area (Figures 6a and 6b), with only few unlabeled septae between the main fields of the somatosensory representation, forming a pattern similar to that detected with CO histochemistry. In coronal sections, 5-HT-immunoreactive fibers formed two dense continuous bands, one in layer VI and the other in layer IV, instead of the normal puffs in layer IV, with light labeling in layer VI (Figures 6c and 6d).

Pharmacological Manipulation of Monoamines in Tg8 Mice

To determine whether the increased amounts of 5-HT or NA present during postnatal development in Tg8 mice (Cases et al., 1995) were responsible for the cortical abnormalities in S1, we administered inhibitors of 5-HT or of NA synthesis from the day of birth (P0) until P6.

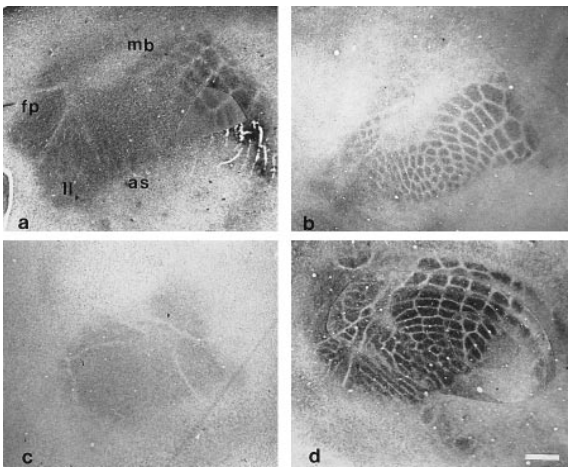

Figure 7. Alteration of the Barrel Field Pattern Produced by Early Inhibition of MAOA in Normal Mice and Reversal of the Barrelless Phenotype in Tg8 Mice with PCPA

Effects of different pharmacological treatments in normal (a and b) and Tg8 (c and d) mice on the formation of the barrel field, viewed on tangential, CO-stained sections.

(a) A P10 normal mouse in which clorgyline was administered from P0 to P6. Note the fusion of the barrels in most of the representation, although a few normal barrels corresponding to large mystacial vibrissae are present.

(b–d) All mice were treated from P0 to P6 and sacrificed at P8. The development of barrels is normal in C3H mice treated with PCPA (b). α-MPT fails to restore the normal barrel field in Tg8 mice (c). PCPA treatment restores a normal-appearing barrel field in Tg8 mice (d). Bar, 0.5 mm.

To reduce 5-HT amounts, we administered parachlorophenylalanine (PCPA). Hardly any 5-HT-labeled fibers were visible in the cerebral cortex when pups were sacrificed 6 hr after the last injection; at 48 hr after the last PCPA administration, high levels of 5-HT immunostaining were recovered in Tg8 but not in C3H pups. Because PCPA has been reported also to reduce catecholaminergic fibers (Chen et al., 1994), we carried out tyrosine hydroxylase immunolabeling, which appeared to be normal. Except for their lack of staining with 5-HT, barrels formed normally throughout S1 in PCPA-treated C3H mice (Figure 7b). In Tg8 mice, PCPA treatment reversed the cortical abnormalities completely. As demonstrated with CO staining (Figure 7d) and with 5-HT and tenascin immunostaining, barrels were now visible throughout S1, delineating the normal pattern. The normal characteristics of the barrels, as determined from Nissl and CO staining, persisted into adulthood.

To reduce catecholamine levels, α-methylparatyrosine (α-MPT), an inhibitor of tyrosine hydroxylase, was administered during the same developmental time period, at a dosage reputed to cause profound reductions of brain catecholamine levels (Hallman and Jonsson, 1984). This treatment, which did not reduce 5-HT immunostaining, had no visible effect on the formation of the barrel field; cortical barrels formed normally in α-MPT-treated C3H mice and failed to develop in α-MPT-treated Tg8 mice (Figure 7c).

Thus, only the reduction of the amount of 5-HT in the brain during the first postnatal week reverses the abnormal cortical phenotype in Tg8 mice.

Pharmacological Inhibition of MAOA in Normal Mice

To determine further whether transient inactivation of MAOA is sufficient to cause alterations of the barrel field, we administered clorgyline, a specific irreversible inhibitor of MAOA (Weyler et al., 1990), to normal pups during the first postnatal week. Measurements of MAOA activity on brain homogenates of mice at P3 showed that this treatment had suppressed any measurable enzymatic activity; increased 5-HT immunostaining was observed throughout the brain, comparable to that observed in Tg8 mice. We observed a disruption of the barrel field in the rostral part of the somatosensory map (anterior snout, lower lip, hindlimb, and forelimb), with a partial blurring or disorganization of the PMBSF (Figure 7a) that was more or less pronounced in different individuals. Abnormal cytoarchitecture of layer IV was found on Nissl-stained coronal sections. As in Tg8 mice, normal CO patterns were observed in the thalamic and brainstem trigeminal relays. The observed disruptions were permanent, being observed whether animals were sacrificed at P10 or as adults.

Thus, inhibition of MAOA activity during the first postnatal week is sufficient to cause alterations in the formation of cortical barrels in S1.

Discussion

The present report provides a clear example, in MAOA-deficient mice (Tg8), of a genetic metabolic deficiency that causes alterations in the formation of the cortical somatosensory map. The causal relationship between the metabolic defect and the cortical phenotype is established by its reversal with pharmacological treatments in the transgenic mice and by its phenocopy in normal mice when MAOA is transiently inactivated during development.

We will first consider the cellular targets that are affected in the Tg8 mice and how these abnormalities appear to be caused by transient excessive amounts of 5-HT in the brain. Finally, we will discuss the relevance of these findings in the general context of the maturation of cortical maps as well as the possible physiological significance of the observed alterations.

Cellular Targets

Our anatomical analysis shows that there is a permanent alteration of the cortical sensory map in the Tg8 mice and that all domains of the primary somatosensory representation, corresponding to both trigeminal and lemniscal afferents, are affected. The alteration is characterized by a lack of patterned distribution of both cells and axonal afferents. This contrasts with the normal organization of the peripheral somatosensory receptors and of their central sensory relays in the brainstem and thalamus, at least as concerns the patterns revealed with CO and cellular stains. Until now, genetic (Welker and Van der Loos, 1986) or lesion-induced (Belford and

Killackey, 1980; Jeanmonod et al., 1981; Jensen and Killackey, 1987) alterations of the cortical barrel field have all been linked to modifications of the peripheral receptors or to interruptions of the ascending trigeminal sensory pathway. The discrepancy observed in our results would thus indicate that the target for the cortical alterations in Tg8 mice does not lie in the early stations of the somatosensory pathway, but rather at its endpoint in the cerebral cortex. Because cortical neurons, glia, and 5-HT and thalamic afferents are all affected, each of these cellular elements could be considered as a primary target in Tg8 mice.

Among these presumptive targets, data in the literature weigh heavily in favor of a primary effect on the thalamocortical projections, which have repeatedly been shown to have an instructive role in barrel formation. Thalamic afferents are indeed the first elements that have a patterned distribution during the perinatal period (Erzurumlu and Jhaveri, 1990; Schlaggar and O'Leary, 1994), closely followed, with a 1 day lag, by 5-HT afferents (Rhoades et al., 1990; Blue et al., 1991). The transient glial boundaries and the aggregation of layer IV neurons form 2–3 days later (Rice and Van der Loos, 1977; Jhaveri et al., 1991). Early postnatal thalamic lesions (Wise and Jones, 1978) prevent the formation of cortical barrels, whereas a normal barrel patterning is observed after early 5-HT lesions (Blue et al., 1991; Bennett-Clarke et al., 1994; Osterheld-Haas et al., 1994) or in tenascin knockout mice (Steindler et al., 1995). Moreover, thalamic lesions reduce the thickness of layer IV (Wise and Jones, 1978), and transection of the infraorbital nerve, which innervates the vibrissae follicles, causes a fusion of the main barrel rows in the PMBSF (Jensen and Killackey, 1987; Rhoades et al., 1990). These two abnormalities are very similar to what we observed in the Tg8 mice. Thus, despite the normal histological organization of the thalamic cell bodies with respect to the formation of thalamic barreloids in the VB, we suggest that the cortical alterations in Tg8 mice could result from a primary abnormality in the formation of the thalamocortical arbors.

In studying these thalamocortical projections in Tg8 mice, we observed that their crude topographic order seemed to be maintained, although the clustered distribution of axons and terminals within layer IV had disappeared. Topographic order in the thalamocortical projection appears to be established very early during embryonic life, when thalamic axons leave the thalamus and before they reach the cortex (Agmon et al., 1995; Molnar and Blakemore, 1995). During the first postnatal day, thalamocortical axons, which are still residing in the deep cortical layers, delineate the main fields of the sensory representation and rapidly develop barrel-like patterns (Schlaggar and O'Leary, 1994). From layer VI, they grow into layer IV, following radial trajectories (Agmon et al., 1993). It will be important to determine whether the observed abnormalities in Tg8 mice concern the early patterning in deep layers or the subsequent refinement of the map and the ingrowth of fibers into layer IV. The observation of rough periphery-related patterns such as barrel rows in some Tg8 mice, as well as the recovery of a normal somatosensory map when pharmacological manipulation of 5-HT was started 5 hr after birth, provides indirect support for the second hypothesis.

Role of MAOA Inhibition during Early Postnatal Development

The critical period for the formation of cortical barrels, when alterations of the cortical patterns (e.g., fusion of the barrels) can be induced by lesions of the peripheral receptors or of the sensory trigeminal relays, has been determined to be limited to the first 3–4 days of life (Belford and Killackey, 1980; Jeanmonod et al., 1981). In the present study, our pharmacological manipulations with clorgyline in normal mice support the notion that, in order to induce phenotypic changes somewhat similar to those observed in Tg8 mice, the inhibition of MAOA must occur during the first postnatal week. Exposure to clorgyline only during the embryonic period, or after the fourth postnatal day (data not shown), did not result in structural changes in S1. This pharmacological critical period overlaps with the ontogenic critical period. The cortical changes induced by early treatment with clorgyline in normal mice were not as marked as those observed in Tg8 mice, because barrels were present in the PMBSF. This incomplete phenotype may be due to technical difficulties in obtaining a rapid and permanent inactivation of the enzyme because of the important de novo synthesis of MAOA in young pups (Samsa et al., 1979).

Role of Excessive Amounts of 5-HT during Early Postnatal Development

MAOA catalyzes the deamination of both 5-HT and NA, and its deficiency causes increased levels of both amines in the brain, each being possibly involved in the alteration of morphogenetic events. However, biochemical analyses in Tg8 mice have indicated that during the first posnatal week, the MAOA deficiency has much larger effects on 5-HT than on NA in the brain; 5-HT amounts are increased by 700%–900%, whereas NA levels are increased by only 35%–70% (Cases et al., 1995). This suggested that the transient increase in brain 5-HT amounts may be the principal culprit for the observed structural modifications. Pharmacological manipulations of Tg8 mice during the first postnatal week support this contention, since the inhibition of catecholamine synthesis with α-MPT caused no change of the cortical phenotype, whereas reduction of 5-HT synthesis by administration of PCPA restored the normal development of the barrel field.

Possible Mechanisms and Significance in the Normal Process of Sensory Map Formation

An important issue raised by our results is whether the disruption of the barrel field, caused by the excessive 5-HT concentrations in the brain, reflects a completely abnormal developmental process or the exaggeration of normal effects of 5-HT on the development of the somatosensory map.

High concentrations of 5-HT in the brain may cause detrimental effects on its development, either directly or via the production of other hormones or neurotransmitters. For instance, owing to MAOA deficiency, 5-HT could accumulate in abnormal locations, in particular in cell populations poor in MAOB, and cause disregulation in these cells. We have previously shown that 5-HT accumulates in catecholaminergic neurons of the locus coeruleus, substantia nigra, and ventral tegmental area (Cases et al., 1995), where it could affect the normal function of the cortically projecting neurons.

There are solid grounds to support the contention that 5-HT could be involved in the normal process of S1 formation. Developmental studies have indicated that 5-HT axons form early, transient periphery-related patterns in S1, tightly related to the pattern of thalamic afferents (Fujimiya et al., 1986; D'Amato et al., 1987; Rhoades et al., 1990; Blue et al., 1991). During the same time period, 5-HT_{1B} receptors are present on the thalamocortical axons (Bennett-Clarke et al., 1993). Although further experimental data failed to demonstrate that these 5-HT afferents have an instructive role in the formation of the barrel field (Blue et al., 1991; Bennett-Clarke et al., 1994; Osterheld-Haas et al., 1994; and confirmed in the present study), they indicated that 5-HT could have a trophic or modulatory effect. Early 5-HT depletion delays the emergence of the thalamocortical periphery-related patterns (Blue et al., 1991) and the maturation of cortical layers (J. P. Hornung, personal communication), with a corresponding prolongation of the critical period for plastic changes in the barrel field cortex (Osterheld-Haas et al., 1994). Furthermore, Bennett-Clarke et al. (1994) reported that when 5-HT fibers are lesioned on the day of birth, there is a 20%–30% decrease in the size of individual barrels and a reduced tangential extent of thalamocortical arbors. It is interesting to relate this finding to the present observation in Tg8 pups, exposed to high 5-HT levels, of a 20% increase in the tangential extent of S1, with VB thalamocortical fibers filling interbarrel septae that are normally devoid of such arbors. These opposed effects could be taken as an indirect indication that 5-HT has an influence on the growth of thalamic axons in S1. However, three-dimensional reconstructions of single thalamic fibers and terminal arbors would be necessary to determine whether a lack or an excess of 5-HT has an effect on their intracortical growth.

If, as discussed above, 5-HT is implicated in the development of the barrel field, its mechanisms of action still remain speculative. In vivo, 5-HT is known to modulate neurite extension and axonal branching patterns. In vitro, 5-HT agonists may either induce the arrest of growth cones and stop axon elongation (Goldberg et al., 1991) or promote growth and differentiation (Chubakov et al., 1986; Riad et al., 1994). Thus, the effects of 5-HT on growth vary according to the cell type, possibly in relation to the 5-HT receptor subtypes that are activated. Preliminary studies of dissociated embryonic thalamic neurons in culture suggest that 5-HT affects the growth of this type of neuron (R. B. Lotto and P. G., unpublished data). It could affect growth, for instance, by enhancing the production of neurotrophic factors (Whitaker-Azmitia and Azmitia, 1989; Whitaker-Azmitia et al., 1990) or by reducing the production of factors that inhibit growth.

The effects of 5-HT may also be related to the modulation of neural activity in the developing cortex. During the first 2 weeks of postnatal life, 5-HT_{1B} receptors are expressed on the glutamatergic thalamocortical neurons (Bennett-Clarke et al., 1993) and mediate strong presynaptic inhibitory effects upon thalamocortical transmission (Rhoades et al. 1994). In Tg8 pups, 5-HT_{1B} receptors were still detectable in the cortex. Thus, excessive amounts of 5-HT could result in a functional silencing of the thalamic neurons, which would be reversed when 5-HT levels were normalized. Until now, experimental data have not demonstrated a clear effect of neural activity in the formation of the barrel field (reviewed in O'Leary et al., 1994); early postnatal blockade of sensory nerve impulses with tetrodotoxin (Chiaia et al., 1992; Henderson et al., 1992) or inhibition of the NMDA receptors (Schlaggar et al., 1993) does not alter the formation of the normal barrel field pattern, although the latter was shown to influence plastic changes of the barrels during the critical period. However, the timing of these electrical blockade experiments may have been too tardy, since the complete lack of NMDA receptor, in knockout mice, prevents the early postnatal formation of whisker-related patterns in the brainstem trigeminal complex (Li et al., 1994).

Possible Physiological Significance

The abnormalities of cortical maturation described here could underlie some of the behavioral alterations of the Tg8 mice. As adults, male mice were found to be unusually aggressive. Both males and females had reduced activity in the open field and were clumsy and hesitant in the beam-walking test (Cases et al., 1995). These latter behaviors could be relevant to the cortical somatosensory abnormalities observed, since the barrel field cortex appears to be important for exploration and sensorimotor integration (Welker, 1976; Hurwitz et al., 1990). However, the permanent metabolic deficiency of monoamines (Cases et al., 1995) could also contribute to this abnormal behavior. Behavioral analyses of Tg8 mice in which the histological alterations have been reversed by early pharmacological treatments, as demonstrated in the present experiments, will help to resolve this issue.

Our findings could be relevant to several human states in which the MAO genes were shown to be altered. In some patients with atypical Norrie's disease, characterized by mental retardation, a genetic deletion has been shown on the X chromosome, involving the *MAOA* and *MAOB* genes (Sims et al., 1989). Closer to the deficiency found in the Tg8 mutants, Brunner et al. (1993) described a point mutation on the *MAOA* gene that is associated with mild mental retardation and increased aggressiveness in male patients. Although no structural equivalent of the rodent barrel field has yet been described in primates, the mechanisms implicated in the formation and maintenance of the cortical maps could be similar, and one could speculate that an altered cortical development exists in these human patients.

Experimental Procedures

Design of Transgenic Animals

Transgenic mice were obtained by microinjection of an interferon-β minigene (Cases et al., 1995) into C3H/HeJ fertilized eggs. In one line characterized by abnormal behavior of mouse pups, the transgene was shown to be integrated into the gene encoding MAOA, causing the deletion of exons 2 and 3 and resulting in the complete lack of MAOA activity (Cases et al., 1995). Normal C3H/HeJ mice were used as controls (C3H). Animals were sacrificed at P7 or P8 (P0 being the day of birth) and as adults (2–3 months old).

Immunocytochemistry and CO Histochemistry

Adult and P7 or P8 mice were perfused with 4% paraformaldehyde for 10–15 min. The dissected brains were immersed in the same fixative for 4–24 hr while cryoprotected (30% sucrose in 0.1 M phosphate buffer; pH 7.4). In most cases, one hemisphere was separated from the brain and flattened between two glass slides. This hemisphere was tangentially cut on a microtome into 40 μm sections. The other hemisphere, with the brainstem, was serially cut in the coronal plane into 30 μm thick frozen sections. Alternate series of sections were used for CO, immunostaining, and Nissl.

CO activity was revealed as described by Wong-Riley and Welt (1980). In brief, sections were sequentially incubated in phosphate buffer (0.1 M; pH 7.4) with 10% sucrose; 0.2% cobalt chloride (10 min); phosphate-buffered sucrose; 0.007% cytochrome C, 0.002% catalase, 0.02% dimethylsulfoxide, and 0.05% diaminobenzidine in phosphate-buffered sucrose (all products from Sigma); and phosphate buffer.

For immunostaining, antibodies to 5-HT (rat monoclonal from Seralab [1:2000–1:4000] or polyclonal from INC [1:10000–1:15000]) and tyrosine hydroxylase (rabbit polyclonal [1:500]; the kind gift of Annette Vigny) were diluted in 0.02 M phosphate-buffered saline with 0.2% gelatin and 0.25% Triton X-100 (PBS+). Sections were rinsed in PBS+, incubated overnight at room temperature in the primary antibody, rinsed 4 times in PBS+, incubated 2 hr in 1:200 biotinylated secondary antibody (anti-rabbit from Sigma and anti-rat from Amersham), rinsed twice in PBS+, incubated 2 hr in 1:400 streptavidin peroxidase complex (Amersham), rinsed in Tris-buffer (0.05 M; pH 7.8), and revealed in 0.02% diaminobenzidine, 0.003% H_2O_2, 0.6% nickel ammonium sulfate. The mouse monoclonal anti-tenascin antiserum (Sigma; diluted 1:5000) was revealed following the same protocol, except that Triton X-100 was omitted from the PBS+.

Histology–Paraffin Series

Two adult Tg8 and two C3H mice were perfused with 1% paraformaldehyde, 1% glutaraldehyde and postfixed for 5 hr. The brains and whisker pads were embedded in paraffin wax. Serial 7.5 μm thick brain sections were then Nissl stained (cresyl violet and thionin). Sections from the whisker pads were stained with the Bodian-Luxol stain.

Pharmacological Treatments

Preliminary experiments with different dosages of clorgyline (N-methyl-N-propargyl-3-(2,4-dichlorophenoxy) propylamine) indicated that the necessary dosage schedule to obtain an effect was 10 mg/kg/8 hr. MAOA activity was determined as described by Wu et al. (1993), using ^{14}C-labeled serotonin as a substrate. Clorgyline (10 mg/kg/8 hr) or saline was administered subcutaneously from P0 to P6 (n = 5). Mice were sacrificed during the second postnatal week (P10) or as adults (n = 2), and the brains (one side cut tangentially, the other coronally) were stained for CO, Nissl, and 5-HT immunocytochemistry. PCPA was subcutaneously injected daily (300 mg/kg) from P0 to P6 in Tg8 (n = 3) and in C3H (n = 2) pups. α-MPT (300 mg/kg) was injected from P0 to P6 into Tg8 pups (n = 3). Animals were sacrificed at P8, 48 hr after the last injection: one hemisphere was flattened and stained for CO and 5-HT, and the other hemisphere was cut coronally; alternate sections were used for 5-HT, tenascin, and tyrosine hydroxylase immunocytochemistry, and for CO staining.

Dextran–Biotin Injections in the Thalamus

Adult female mice (six Tg8 and ten C3H) were injected with dextran–biotin (10,000 MW; Molecular Probes). Glass micropipettes (30–40 μm tip diameter) were filled with 2% dextran–biotin. Mice were placed in a David Kopf stereotaxic apparatus with a mouse adaptator. The pipettes were lowered with micromanipulators into the VB. Identical coordinate positions were used in the Tg8 and C3H mice: these injections, one on each side, were located at two different positions of the thalamus (first coordinate position: −2 anterior, 1.3 lateral, and −3.5 ventral to the bregma; second coordinate position: −3 anterior, 2 lateral, and −3 ventral to the bregma). These injections involved the VB and occasionally the posterior nuclear group. Dextran–biotin was ejected by passing 7 μA positive currents (7 s on, 7 s off) for 10 min. Mice were perfused for 6–7 days after the injection. The brains were sectioned coronally (30 μm), and biotin was revealed with the streptavidin peroxidase complex as described above in the immunostaining protocol. Series of sections were counterstained with 1% methyl green in 70% ethanol or with CO to localize the cortical layers and the site of the thalamic injections.

Cyanopindolol Labeling

Brains of 7-day-old C3H (n = 3) and Tg8 (n = 3) pups were directly frozen in isopentane (−40°C) and cut into 10 μm thick cryostat sections in the coronal or tangential plane. Sections were collected onto 0.5% gelatin-coated slides. After a preincubation in ice-cold buffer (50 mM Tris-HCl, 2.5 mM $MgCl_2$, 10 μM pargyline, 0.1 μM 8-OH-DPAT, 30 μM isoproterenol; pH 7.5) for 10 min, sections were incubated in the same buffer containing 100 pM [^{125}I]cyanopindolol at room temperature for 1 hr. After incubation, sections were washed twice in cold buffer and dipped once in cold distillated water. Dry slides were then apposed to Hyperfilm-^{3}H (Amersham) overnight.

Analysis

Measurements of the cortical thickness of layer IV were obtained from 24 coronal sections (paraffin embeded material) taken at the level of the PMBSF, in two C3H and two Tg8 3-month-old mice. In P8 mice, the complete map of the barrel field was reconstructed from camera lucida drawings and from micrographs of serial tangential sections stained for CO. This map was redrawn on transparent foils, and the area of the external contour of S1 was measured using an image analyzer.

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